



Cronfa - Swansea University Open Access Repository

This is an author produced version of a paper published in:

Journal of Animal Science

Cronfa URL for this paper:

<http://cronfa.swan.ac.uk/Record/cronfa20697>

Paper:

Bromfield, J., Santos, J., Block, J., Williams, R. & Sheldon, I. (2015). PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: Uterine infection: Linking infection and innate immunity with infertility in the high-producing dairy cow.

Journal of Animal Science, 0(0), 0

<http://dx.doi.org/10.2527/jas.2014-8496>

This item is brought to you by Swansea University. Any person downloading material is agreeing to abide by the terms of the repository licence. Copies of full text items may be used or reproduced in any format or medium, without prior permission for personal research or study, educational or non-commercial purposes only. The copyright for any work remains with the original author unless otherwise specified. The full-text must not be sold in any format or medium without the formal permission of the copyright holder.

Permission for multiple reproductions should be obtained from the original author.

Authors are personally responsible for adhering to copyright and publisher restrictions when uploading content to the repository.

<http://www.swansea.ac.uk/library/researchsupport/ris-support/>

RUNNING HEAD: Uterine infection and infertility in dairy cows

**Uterine infection: Linking infection and innate immunity with infertility in
the high producing dairy cow¹**

J.J. Bromfield^{*†2}, J.E.P Santos^{*†}, J. Block[†], S.R. Williams^{*‡} and I.M. Sheldon^{§3}

^{*}D. H. Barron Reproductive and Perinatal Biology Research Program; [†]Department of Animal Sciences, University of Florida, Gainesville 32611; [‡]Department of Obstetrics and Gynecology, College of Medicine, University of Florida, Gainesville 32606; and [§]Institute of Life Science, College of Medicine, Swansea University, Singleton Park, Swansea, SA2 8PP United Kingdom

¹ Based on a presentation at the Physiology and Endocrinology Symposium titled

“Reproductive success in ruminants: A complex interaction between endocrine, metabolic and environmental factors” at the Joint Annual Meeting, July 20-24, 2014, Kansas City, MO.

² Corresponding author: jbromfield@ufl.edu

³ I.M. Sheldon was funded by the U.K. Biotechnology and Biological Sciences Research Council (www.bbsrc.ac.uk grant number BB/K006592/1).

ABSTRACT: Uterine contamination with bacteria is ubiquitous in the postpartum dairy cow. Nearly half of all postpartum dairy cows develop clinical disease resulting in metritis and endometritis, which causes depressed milk production and infertility. The causative links between uterine infection and infertility include a hostile uterine environment, disrupted endocrine signaling, and perturbations in ovarian function and oocyte development. In this review we consider the various mechanisms linking uterine infection with infertility in the dairy cow; specifically, 1) innate immune signaling in the endometrium, 2) alteration in endocrine signaling in response to infectious agents, and finally 3) impacts of infection on ovarian function and oocyte/ follicle development. Normal ovarian follicle and oocyte development requires a series of temporally and spatially orchestrated events. However, several of the cellular pathways required for ovarian function are also used during the innate immune response to bacterial pathogens. We propose that activation of cellular pathways during this immune response has a negative impact on ovarian physiology, which is manifest as infertility detected after the clearance of the bacteria. This review highlights how new insights into infection and immunity in cattle are linked to infertility.

Key words: cow, immunity, infection, oocyte, ovary, uterus

INTRODUCTION

The uterine mucosal environment is protected from pathogenic bacterial infiltration by physical anatomical barriers and active molecular mechanisms. However, in comparison to non-dairy cattle breeds, high-milk-yield dairy breeds such as the Holstein-Friesian are prone to uterine bacterial contamination when these mechanisms fail, primarily following parturition. In

the dairy cow, parturition generates considerable tissue damage to the endometrium and cervix, which gives results in the failure of the anatomical barriers to prevent ascending uterine bacterial infiltration. While bacterial infections are normally cleared within 3 to 5 wk of parturition, many cows display signs of impaired fertility, including reduced conception, lower submission rates, and increased calving to conception intervals, and these signs are temporally after the resolution of the signs of uterine disease (Borsberry and Dobson, 1989; McDougall, 2001; LeBlanc et al., 2002). The innate immune response to bacteria is key to rapidly clearing infection (Herath et al., 2006; Davies et al., 2008; Cronin et al., 2012; Turner et al., 2014). Recruitment of hematopoietic immune cells, and the inflammatory response including secretion of cytokine and chemokines, all combine to clear the bacterial infection and restore hemostatic function of the endometrium (Sheldon and Roberts, 2010). However, evidence is emerging that these inflammatory events have long-term consequences on the fertility of dairy cows by negatively impacting endocrine signaling, uterine homeostasis, and ovarian function.

POST-PARTUM DISEASE IN THE DAIRY COW

Under normal circumstances, the anatomical barrier of the cervix and various mucosal mechanisms including mucus, intact epithelium, and antimicrobial agents protect the uterine environment from pathogens ascending the reproductive tract from the vagina. When these barriers are breached during parturition or insemination, bacterial pathogens can rapidly invade the uterus to establish infection, and this can result in clinical disease if cellular and humoral defense mechanisms are overwhelmed (Bondurant, 1999). Indeed, during and after parturition, bacteria readily ascend the female genital tract into the uterus. While the majority of bacterial infection occurs in the postpartum animal, venereal transmitted pathogens including *Tritrichomonas foetus* and *Campylobacter fetus* can cause moderate persistent inflammation of

the uterus and result in increased pregnancy losses (Corbeil et al., 1975; Schurig et al., 1975; Parsonson et al., 1976; Skirrow and BonDurant, 1990). Similarly, hygiene standards at AI have also been shown impact bacterial contamination and to reduce the likelihood of pregnancy (Bas et al., 2011). It is estimated that about 40% of dairy cows develop clinical metritis following parturition, characterized by vaginal discharge of a brown foul-smelling watery discharge from the uterus, fever, reduced milk yield and, in severe cases, toxemia (Markusfeld, 1987; Zwald et al., 2004). Most cases of metritis are resolved within 14 d of diagnosis with or without the use of antimicrobial therapy (Chenault et al., 2004). However, approximately 20% of the cows develop endometritis beyond 21 d postpartum, with a persistent purulent discharge from the uterus. Terminology defining endometritis is beginning to change as purulent vaginal mucus is not always correlated with the number of neutrophils detected in cytology samples collected from the surface of the endometrium by cytobrush (Dubuc et al., 2010). Furthermore, cytological endometritis has emerged as a problem of importance for dairy cattle reproduction because of reduced pregnancy per insemination and extended interval to pregnancy (Vieira-Neto et al., 2014). Animals suffering cytological endometritis present a persistent inflammatory uterine environment in the absence of clinical symptoms. The definitions of disease are set out in reviews (Sheldon et al., 2006; Sheldon et al., 2009). However, recent assessments of the literature suggest some discrepancies remain between research teams as to the best practice for clinical diagnosis of both endometritis and metritis (Sannmann et al., 2012; de Boer et al., 2014). The financial impact of uterine disease in dairy cows has been estimated at approximately \$650 million in the U.S., with costs stemming from clinical treatment, lost milk production, culling for failure to conceive and maintenance of replacement animals (Sheldon et al., 2009).

While parturition is considered the event leading to uterine infection, several risk factors

are associated with increasing the risk of uterine diseases. These include factors associated with uterine damage, such as dystocia, retained fetal membranes, twins, and stillbirth (Potter et al., 2010; Giuliadori et al., 2013). Parity has also been associated with uterine disease, with the highest odds of developing metritis associated with either first, or greater than or equal to third parity. It is surmised that primiparous animals are at increased risk of dystocia while older third-parity animals are at high risk of retained fetal membranes, both resulting in uterine damage (Bruun et al., 2002). The dairy cow experiences a period of negative nutrient balance following parturition because of the high dietary demands for energy for milk synthesis. Energy homeostasis and metabolism are closely associated with the effectiveness of the immune system to combat infections (Mathis and Shoelson, 2011). Recent studies have given weight to the argument that cows with markers of more exacerbated tissue catabolism because of negative energy balance are more likely to develop uterine diseases (Silvestre et al., 2011; Giuliadori et al., 2013; Ribeiro et al., 2013).

In many instances, uterine infection and disease is common and treatment is relatively straightforward. However, the consequences of infection and inflammation of the reproductive tract persist beyond the resolution of the clinical process, with marked depression in reproductive performance (Borsberry and Dobson, 1989; LeBlanc et al., 2002; Kasimanickam et al., 2004; Sheldon et al., 2009). Cows with clinical disease show a longer interval to estrus, irregular ovarian cycles, a prolonged postpartum luteal phase, delayed onset to ovarian cyclicity, and ultimately failure to conceive (Ribeiro et al., 2013). Compared with normal cows, endometritis results in a 1.7-fold increase in the culling rate of animals (LeBlanc et al., 2002). Treatment of cows with metritis uses routine administration of systemic antimicrobials, some of which have no milk discard requirements (Chenault et al., 2004), although the benefits of their use to

improve reproductive performance remain to be demonstrated (Galvao et al., 2009). More recently, attempts have been made to produce vaccines to prevent metritis and preliminary results are encouraging (Machado et al., 2014). However, after the resolution of clinical disease, cows still have reduced pregnancy/ AI (Borsberry and Dobson, 1989; LeBlanc et al., 2002; Kasimanickam et al., 2004; Sheldon et al., 2009). The mechanistic reasons behind continued infertility following resolution of infection (metritis), or resultant inflammation (endometritis), remains to be elucidated.

PATHOGENIC AGENTS INVOLVED IN UTERINE INFECTION

Microbial contamination of the uterus occurs shortly after parturition by a number of opportunistic bacteria including *Escherichia coli*, *Trueperella pyogenes*, and the anaerobes *Prevotella sp*, *Fusobacterium necrophorum*, and *Fusobacterium nucleatum*. The first bacterial pathogen to colonize the upper reproductive tract following parturition involved in uterine disease is *E. coli* (Williams et al., 2007); and endometrial specific strain of *E. coli*, termed endometrial pathogenic *E. coli* (**EnPEC**) are distinct from gastro-intestinal and extra-intestinal pathogenic *E. coli* (Sheldon et al., 2010). Analysis of this specific strain revealed a surprising lack of virulence factors associated with EnPEC compared with pathogenic enteric and extra-intestinal pathogenic *E. coli*. However, EnPEC possess an increased ability to adhere and invade endometrial cells than other *E. coli* strains. Characterization of other metritis associated bacteria, including some *E. coli* strains, have shown specific virulence factor expression like FimH (Sheldon et al., 2010; Bicalho et al., 2012). Endometrial pathogenic *E. coli* induce an endometrial inflammatory response due to the presence of the cell wall component lipopolysaccharide (**LPS**; i.e., an endotoxin). Endometrial specific *E. coli* have now been sequenced but the mechanism by which these bacteria preferentially establish disease in dairy

cows is unclear beyond LPS initiated inflammation (Goldstone et al., 2014b). It is interesting to note that recently the degree to which *E. coli* is associated with uterine disease has come into question. Pyrosequencing for the microbiome of the uterus revealed a surprising absence of *E. coli* at 35 DIM, while other studies have suggested a limited association with the presence of *E. coli* at this time point with uterine disease or infertility (Bicalho et al., 2010; Machado et al., 2012). However, it is important to realize the distinction between the presence of uterine *E. coli* at 35 days in milk (**DIM**) and the importance of uterine *E. coli* shortly after parturition where an association with uterine disease and infertility exists (Dohmen et al., 2000; Mateus et al., 2002; Sheldon et al., 2002; Williams et al., 2007; Sheldon et al., 2010; Prunner et al., 2014; Wagener et al., 2014).

Infection with *T. pyogenes* (formally *Arcanobacterium pyogenes*) is associated with the most severe cases of uterine inflammation in dairy cattle at day 26 or 40 postpartum (Bonnett et al., 1991; Prunner et al., 2014). *Trueperella pyogenes* elicits an inflammatory response in endometrial explants, increasing the inflammatory mediators IL-1 β , IL-6, IL-8 and PGF_{2 α} (Miller et al., 2007; Amos et al., 2014). However, much of the virulence of *T. pyogenes* is associated with the organism's secretion of a cholesterol dependent cytolysin, pyolysin (**PLO**), which causes osmotic death of host cells. Exposure of endometrial stromal cells to PLO potently elicits cytolysis, although endometrial epithelial cells appear resistant to PLO-mediated lysis, probably due to the lower cholesterol content of epithelial than stromal cells (Amos et al., 2014). The differential cellular susceptibility to PLO reflects the observations that uterine damage is required for infection to cause disease, particularly when the protective epithelium is disrupted following parturition. Endometritis-causing *T. pyogenes* have now been fully sequenced and are highly similar amongst cows with uterine disease, and can produce experimentally induced

infection resulting in clinical signs of endometritis with a purulent discharge in the uterus and vagina (Amos et al., 2014; Goldstone et al., 2014a).

The above mentioned microbes dominate the literature regarding uterine disease, but it is important to consider the presence of lesser studied bacterial species associated with disease. The anaerobes *Prevotella sp*, *F. necrophorum*, and *F. nucleatum* have all been associated with severe uterine disease in cattle and appear to aid the pathogenesis of both *E. coli* and *T. pyogenes* (Olson et al., 1984). For example, *F. necrophorum* produces a leukotoxin which inactivates and kills leukocytes required to clear an infection (Narayanan et al., 2002). *Prevotella melaninogenica* has been shown to produce substances which inhibit phagocytosis of bacteria and induce the production of factors by the host immune system to cause tissue destruction (Jones and Gemmell, 1982; McGregor et al., 1986). It is interesting to note that other bacterial strains are also present in the uterus and are not associated with uterine disease, such as *Staphylococci* and *Streptococci* (Williams et al., 2005). Furthermore, a wide variety of microbes can be identified in the postpartum uterus by molecular techniques, although their roles remain unclear (Machado et al., 2012).

TOLL-LIKE RECEPTOR SIGNALING

Bacteria utilize specialized virulence factors to cause tissue damage and promote disease, which leads a host response to these bacteria directed by the innate immune system, including antimicrobial peptides, complement, and the Toll-like receptor (TLR) family. It is known that antimicrobial peptides and complement play a critical role in the initiation of inflammation and clearance of microbes from the uterus of cows (Bondurant, 1999); however, this review will focus on the role of TLR. Since Hoffman and Beutler identified the importance of Toll in flies and TLR in mammals for initiating the immediate response to pathogens (Lemaitre et al., 1996;

Poltorak et al., 1998), the field of innate immunity has lavished a great deal of attention on this family of receptors and the role they play in disease.

In the cow there are 10 members of the TLR family (TLR1 to 10), each with the capability to bind specific conserved microbial components, although TLR10 has yet to be assigned a ligand. Each TLR has a specific cellular location dependent on the ligand to which it binds; TLR 3, 7, 8 and 9 are intracellular, whereas the remainder are principally cell surface receptors. The cell surface receptors mainly bind bacterial lipids, often bacterial cell wall components, whereas intracellular receptors bind nucleic acids, indicating a highly evolved mechanism to detect the presence of microbial agents dependent on their type and pathogenesis (Beutler, 2004). Of relevance to uterine disease, bacterial LPS binds to TLR4 in conjunction with the co-receptors lymphocyte antigen 96 (**LY96**, also known as **MD-2**) and cluster of differentiation (**CD**) 14, whilst bacterial lipopeptides are bound by TLR2 in concert with TLR1 or TLR6 (Cronin et al., 2012; Turner et al., 2014). Upon binding the receptor specific ligand, an intracellular signaling cascade results in the expression of inflammatory mediators including the cytokines tumor-necrosis factor (**TNF**)- α , interferon (**IFN**)- γ , IL-1 β , IL-6, and chemokines IL-8 and C-X-C motif chemokine (**CXCL**) 5 required for leukocyte infiltration and clearance of infectious agents (Kawai and Akira, 2010). In dairy cows, all 10 TLR are present in non-pregnant endometrium, whereas expression is variable in the postpartum endometrium but still present (Davies et al., 2008; Herath et al., 2009b) (**Table 1**). Recently it has been described that specific SNP in TLR 2, 4, 6 and 9 have minor associations with uterine disease of dairy cows (Pinedo et al., 2013). These SNP may provide an insight into the variability in disease susceptibility between cows exposed to the same bacteria. However, whilst the mechanisms of infection and disease are being uncovered, the underlying question remains; how do uterine

infections in dairy cows result in infertility after the resolution of infection or disease?

MECHANISMS OF INFERTILITY CAUSED BY UTERINE INFECTION

We hypothesize that there are three main factors linking postpartum uterine infection of dairy cows with infertility, even following clearance of infectious agents. These are: 1) disruption of endocrine signaling and the hypothalamic-pituitary-gonadal axis; 2) negative effects on the ability of the endometrium to support embryo development and implantation; and 3) ovarian dysregulation resulting in reduced oocyte quality (**Figure 1**).

Impact of Uterine Infection on Endocrine Signaling

It is curious to consider the impacts of uterine infection and inflammation on neuro-endocrine signaling due to the spatial distance between the site of infection and the hypothalamus and pituitary on the base of the brain. However, experimental uterine LPS exposure in the postpartum cow or systemic administration in the sheep decreases GnRH secretion by the hypothalamus and reduces LH pulsatility (Peter et al., 1989; Karsch et al., 2002). Furthermore, ovulation is delayed in cows following systemic or intramammary administration of LPS, although GnRH has been used therapeutically to induce normal ovarian cyclicity in these animals (Suzuki et al., 2001; Lavon et al., 2008). The specific mechanisms by which LPS exposure at a distant site impacts hypothalamus-pituitary function has yet to be elucidated, experimental models of systemic LPS administration (above) suggest the possibility of LPS entering the circulation and traveling to the brain, while it is interesting that blockade of TLR4 signaling seems to prevent LPS-induced GnRH-LH signal disruption (Haziak et al., 2014).

The primary impact of disrupting endocrine signaling are the negative consequences on ovarian function. Cows with postpartum uterine infections within two weeks of calving have

reduced circulating estradiol and perturbed prostaglandin signaling resulting in disruption of ovarian cyclicity, extended luteal phases, delayed ovulation, slower follicle growth and increased risk of anovulation (Opsomer et al., 2000; Sheldon et al., 2002; Herath et al., 2007). The changes in follicular estradiol production are a direct result of reduced aromatase activity in granulosa cells due to LPS exposure (Price et al., 2013; Magata et al., 2014). Furthermore, LPS induces a switch of endometrial PG production from luteolytic PGF_{2α} to immune modulatory PGE₂ that likely contributes to the extension of the luteal phase (Herath et al., 2009a).

Endometrial Response to Uterine Infection

Uterine responsiveness to invading microbial pathogens must be rapid and robust to prevent the establishment of uterine infection. Pro-inflammatory genes such as *IL-1α*, *IL-1β*, *IL-6*, *TNFα* and *PTGES* have been shown to be upregulated in the endometrium of animals with persistent endometritis compared to healthy cows (Herath et al., 2009b; Wathes et al., 2009; Fischer et al., 2010). These pro-inflammatory agents increase recruitment of neutrophils and macrophages to combat infection and aid in resolution (Sheldon et al., 2010). The S100 proteins have recently been described to contribute to the inflammatory function of neutrophils, macrophages, and mast cells (Goyette and Geczy, 2011). Endometrial expression of S100A8, S100A9 and S100A12 rapidly increases in response to the inflammatory mediators IL-6 and IL-10 induced by infection (Swangchan-Uthai et al., 2012). In addition, there is increased liver production of serum amyloid A and haptoglobin in cows with uterine disease after parturition (Sheldon et al., 2001). In vitro studies using endometrial explants, or purified endometrial epithelial and stromal cells have shown similar inflammatory responses to *E. coli*, *T. pyogenes* and highly purified bacterial cell wall components, LPS, lipoprotein and peptidoglycan (Borges et al., 2012; Amos et al., 2014; Turner et al., 2014). Induction of proinflammatory mediators IL-

1 β , IL-6 and IL-8 have all been shown to occur in these tissues in a TLR dependent manner depending on the bacterial components utilized (Herath et al., 2006; Cronin et al., 2012; Turner et al., 2014). In combination, increased inflammatory mediators, cellular influx of immune cells and induction of antimicrobial factors all work in concert to combat and clear the active uterine infection. However, as we have alluded to, this response may contribute to the infertility witnessed in cows following the resolution of infection. Indeed, when assessing endometrial expression of the inflammatory mediators *IL-1 α* and *IL-1 β* in cows with infection which became infertile, both mediators are expressed at higher abundance in infertile cows compared with those which remained fertile (Herath et al., 2009b). It is conceivable that an unchecked, excessive endometrial inflammatory response could contribute to infertility in cows following infection. One obvious mechanisms is disruption of the pre-implantation developmental environment. Many embryotrophic factors produced by the oviduct and endometrium are also immune modulators, and as such excessive or inappropriate temporal expression of these factors may perturb embryonic development and negatively impact fertility. Indeed when embryos are cultured in the presence of endometrial fluid from an inflamed uterus, total blasomere number and allocation are negatively impacted (Hill and Gilbert, 2008). Similarly one could surmise that embryo attachment and/or placentation could be equally effected by an inappropriately inflamed uterine environment.

Ovarian Response to Uterine Infection

When considering the mechanisms of infertility following uterine infection, the ovary is a logical target as it contains a vulnerable and finite reserve of oocytes required for subsequent generations; but how does infection at a distant site impact ovarian tissues? As described above, ovarian function is perturbed following infection with reduced estradiol production, delayed

ovulation, retarded follicle growth, and extended luteal phases. Beyond the direct effects of endocrine dysregulation on the ovary by altered LH patterns and shifts from endometrial PGF_{2α} to PGE₂ synthesis, cellular and molecular pathways within the ovarian follicle are also affected in cows suffering uterine infection. Key to the changes seen in the ovary is the presence of LPS within the follicular fluid of diseased cows (Herath et al., 2007). We are now beginning to understand the mechanisms by which the follicular environment, which is free of immune cells, can contribute to infertility in dairy cows.

Granulosa cells possess the molecular machinery required for detection of bacterial components, TLR, CD14 and MD-2. In addition, granulosa cells exposed to the bacterial components LPS or peptidoglycan mount an acute inflammatory response by increased production of inflammatory mediators IL-1β, IL-6, IL-8 and TNFα (Herath et al., 2007; Bromfield and Sheldon, 2011; Price et al., 2013; Price and Sheldon, 2013). The granulosa cell inflammatory response to LPS is initiated by TLR4 and intracellular signaling occurs through rapid phosphorylation of the extracellular-signal-regulated kinases (**ERK**) and p38 kinase pathways (Bromfield and Sheldon, 2011; Price et al., 2013). Estradiol production by granulosa cells is reduced following LPS exposure by reducing aromatase expression, however the mechanism of aromatase reduction is unclear (Herath et al., 2007). Of paramount interest is the finding that oocyte maturation is also perturbed in the presence of LPS (Bromfield and Sheldon, 2011).

Oocyte maturation occurs spontaneously *in vitro*, developing from the germinal vesicle stage to the metaphase II (**MII**) stage in approximately 24 h. This highly orchestrated development matures both the nuclear and cytoplasmic compartments of the oocyte for the first cellular divisions of the early embryo. Production of IL-6 by cumulus oocyte complexes (**COC**)

289 is increased in response to LPS *in vitro*. Work by our group has shown that oocyte maturation in
290 the presence of LPS at concentrations comparable to those found within the follicle significantly
291 reduces the developmental competence of the oocyte, increasing germinal vesicle breakdown
292 failure, and causing abnormal spindle formation (Bromfield and Sheldon, 2011). Maturation of
293 the COC required for ovulation is also perturbed, with LPS inducing cumulus expansion in the
294 absence of gonadotropin signaling (Bromfield and Sheldon, 2011). In addition, maturation of
295 bovine oocytes in the presence of LPS reduced blastocyst development rate, while embryos
296 cultured in the presence of LPS have no adverse effects on blastocyst development (Soto et al.,
297 2003). In the mouse it has been shown that TLR4 plays a physiological role in COC expansion
298 by binding the endogenous ligand hyaluronan, inducing IL-6 expression and matrix expansion
299 (Shimada et al., 2006; Shimada et al., 2008). However, the mechanism by which LPS reduces
300 oocyte quality is yet to be understood. It is possible that LPS directly influence oocyte
301 development, although it seems more plausible that LPS dysregulates inflammatory mediators
302 required for oocyte development. The physiological importance of cytokines in oocyte
303 development and ovarian function is well established (Espey, 1980; Richards et al., 2008;
304 Spaniel-Borowski, 2011). Immunological factors such as IL-6, colony-stimulating factor 2,
305 leukemia inhibitory factor, IGF-I, TNF α , growth differentiation factor 9, bone morphogenetic
306 protein 15, and epidermal growth factor are all critical to oocyte development and their
307 expression has the potential to be altered during infection (Spicer et al., 1988; Alpizar and
308 Spicer, 1994; Dong et al., 1996; Spicer, 1998; Yan et al., 2001; Molyneaux et al., 2003; Van
309 Slyke et al., 2005; Spicer et al., 2006; Hansen et al., 2014). Redundancies in the mediators
310 between oocyte development and inflammation and alterations in their abundance are more
311 likely mechanisms by which oocyte development is perturbed due to bacterial infection. The

intracellular signaling pathways utilized by these various signaling moieties uses the central phosphatidylinositol-4,5-bisphosphate 3-kinase (**PI3K**)/ protein kinase B (**AKT**) pathway critical for oocyte maturation (Okumura et al., 2002; Van Slyke et al., 2005). It is currently unclear whether the presence of bacterial pathogens alters these intracellular pathways in oocytes, reducing developmental competence of oocytes by disrupting cytoplasmic or nuclear maturation.

The negative effects of LPS on developing oocytes in the dominant follicle explains infertility shortly after infection, but how is long-term infertility explained in animals following uterine infection? We propose that infection also perturbs smaller, developing follicles including primordial stage follicles. Initiation of folliculogenesis is a tightly orchestrated series of molecular and cellular events. Primordial follicles containing an immature oocyte at the dictyotene stage of meiosis are held in a quiescent state by the presence of inhibitor factors including phosphatase and tensin homolog (**PTEN**) and forkhead box O3a (**FOXO3a**) (Castrillon et al., 2003; Reddy et al., 2008; Bao et al., 2011). In vitro culture of cortical ovarian explant results in the unexplained spontaneous activation of the primordial follicle pool to develop primary and secondary follicles. In the presence of LPS primordial follicle activation is increased, resulting in an enlarged pool of primary follicles and a depletion of the primordial follicle reserve (Bromfield and Sheldon, 2013). *In vivo* studies using mice revealed a similar decrease in the primordial follicle reserve in conjunction with an increase in follicle atresia after administration of LPS. The LPS induced activation of the primordial pool occurs in conjunction with loss of PTEN and FOXO3a protein in primordial follicles, but it is unclear if this is causative or resultant of follicle activation. Ovarian explant cultures increase production of inflammatory mediators IL-1 β , IL-6 and IL-8 in response to LPS. It was interesting to note the high basal level of IL-6 production in ovarian explants cultured in control medium, which we

propose is involved in the spontaneous activation of primordial follicles. Assessment of larger pre-antral follicle responses to LPS reveal that these stages, at least *in vitro*, appear to be resistant to the effects of LPS, with no change in estradiol production, or oocyte and follicle growth (Bromfield and Sheldon, 2013). Although the above studies investigate the role of LPS on follicle growth *in vitro*, they suggest that fertility may be affected in both short and long term scenarios with the primordial follicle pool inappropriately activated impacting long-term fertility, while low quality oocytes from dominate follicles may impact short-term fertility. The precise mechanisms by which LPS exposure reduces the primordial follicle pool and oocyte development remain to be elucidated, but we propose that alterations in redundant signaling pathways integral in both immunity and development could play a major role. As in oocyte development, the PI3K/AKT pathway is critical for coordinated recruitment of primordial follicles (Wandji et al., 1996; Fortune, 2003). Similarly, activation of the TLR signaling also uses the central PI3K/AKT pathway to increase production of inflammatory mediators like IL-6, feeding back into the pathway for increased stimulation (Laird et al., 2009). We propose that activation of the TLR4/IL-6 by bacterial pathogens contributes to the inappropriate recruitment of primordial follicles by activation of the same pathway (**Figure 2**). To explore the links between uterine disease and the ovary will require exploiting animal models.

ANIMAL MODELS OF UTERINE INFECTION AND THEIR IMPLICATIONS

In 1929, Nobel laureate August Krogh coined the principle that “for such a large number of problems there will be some animal of choice on which it can be most conveniently studied.” (Krogh, 1929; Albertini, 2011). Krogh’s principle encompasses the development of PCR, using thermo stable *Taq* polymerase from heat labile bacteria (Chien et al., 1976); the study of menstruation in the short tailed fruit bat (Rasweiler and de Bonilla, 1992); and the utilization of

jellyfish green fluorescent protein in cell biology (Chalfie et al., 1994). Unique to animal science, we have ready access to the animals in which the problem is relevant. However, uterine infection in dairy cows shares a number of similarities to puerperal fever and pelvic inflammatory disease (PID) in women. Women who suffer PID, do so as a result of uterine infection usually brought on by sexually transmitted bacterial infections such as *Gonorrhea* and *Chlamydia* (Ross, 2002). Pelvic inflammatory disease causes pain and infertility, and is the leading cause of gynecological hospitalization of women in the developed world (Ross, 2002). Studies in women suggest that PID causes ovarian changes similar to those seen in the dairy cow following uterine infection (Weiner and Wallach, 1974; Margolis, 1976; Bychkov, 1990). In addition, studies of infection and immunity using primary cells from the bovine uterus and ovary are similar to studies using human endometrial and ovarian cells (Sanchotello et al., 1992; Allhorn et al., 2008; Price et al., 2012). The similarities between human PID and bovine uterine disease give us a unique opportunity to understand a disease state pertinent to both agricultural production and human health. For humans, the impact of uterine disease might be particularly important for patients who have unexplained infertility and/or a need for assisted reproduction techniques such as in vitro fertilization where the immune environment requires a balance between physiological (for normally ovarian function) and pathological (to combat bacterial infection) response (Chegini et al., 2002; Li et al., 2006). The availability of bovine oocytes and granulosa cells may provide an opportunity to inform human studies relevant to puerperal fever and PID in women.

Several animal models of uterine infection have been developed with varying degrees of success in reproducing the disease. The mouse has been commonly used by infusing bacteria into the uterus. We have used the mouse as a convenient model by administering LPS intraperitoneally and noted that while primordial depletion occurs, it is likely due to follicle

atresia as opposed to increased primordial follicle activation as in the cow (Bromfield and Sheldon, 2013). In the dairy cow it has long been established that the endocrine status of the animal is key to the development and severity of uterine infection following infusion of *E. coli* and *T. pyogenes*. Induction of the disease at estrus or administration of exogenous estradiol limits the formation of infection, whereas bacterial infusion during the luteal phase or exogenous progesterone administration increases the likelihood of uterine infection persisting (Rowson et al., 1953; Ayliffe and Noakes, 1982). In addition, the structural integrity of the endometrium is important, as noted above physical damage to the epithelial layer seems to be important in establishing disease. Uterine infusion of the *T. pyogenes* virulence factor, PLO without endometrial damage results in no signs of disease (Miller et al., 2007). However, uterine infusion of *T. pyogenes* with mechanical disruption of the endometrium results in uterine disease (Amos et al., 2014). The potential to exploit the dairy cow model of uterine disease has yet to be fully appreciated in regard to human disease, particularly for study of the impacts on the ovary.

SUMMARY AND CONCLUSIONS

Uterine infection and inflammation in the dairy cow causes infertility. However, we are only now beginning to understand the importance of pathological ovarian dysfunction which persists beyond the duration of infection. The effects of infection on uterine and neuro-endocrine homeostasis are well established, however the extended temporal development of the follicle and oocyte lends the ovary to prolonged vulnerability following infectious challenge resulting in perturbations which may not manifest until sometime after disease resolution. Primordial follicle quiescence to ovulation all appear susceptible to perturbation by infections of the reproductive tract, yet little is known about the mechanisms responsible for causing infertility in dairy cows. Further work using a bovine model that recapitulates the bacterial infection and persistent

inflammation of uterine disease is urgently needed to elucidate the pathways and mechanisms responsible for ovarian dysfunction following uterine infection. Appropriate animal models will allow the development of strategies to limit the impact of disease on the dairy industry and provide valuable insight into human health and fertility.

LITERATURE CITED

- Albertini, D. F. 2011. On the matter of Krogh's principle. *J. Assist. Reprod. Gen.* 28:1-2.
- Allhorn, S., C. Boing, A. A. Koch, R. Kimmig, and I. Gashaw. 2008. TLR3 and TLR4 expression in healthy and diseased human endometrium. *Reprod. Biol. Endocrinol.* 6:40.
- Alpizar, E., and L. J. Spicer. 1994. Effects of interleukin-6 on proliferation and follicle-stimulating hormone-induced estradiol production by bovine granulosa cells in vitro: dependence on size of follicle. *Biol. Reprod.* 50:38-43.
- Amos, M. R. et al. 2014. Differential endometrial cell sensitivity to a cholesterol-dependent cytolytic links *Trueperella pyogenes* to uterine disease in cattle. *Biol. Reprod.* 90:54.
- Ayliffe, T. R., and D. E. Noakes. 1982. Effects of exogenous oestrogen and experimentally induced endometritis on absorption of sodium benzylpenicillin from the cow's uterus. *Vet. Rec.* 110:96-98.
- Bao, R. M., K. Hayakawa, M. Moniruzzaman, H. Taketsuru, and T. Miyano. 2011. FOXO3 knockdown accelerates development of bovine primordial follicles. *J. Reprod. Dev.* 57:475-480.
- Bas, S., A. Hoet, P. Rajala-Schultz, D. Sanders, and G. M. Schuenemann. 2011. The use of plastic cover sheaths at the time of artificial insemination improved fertility of lactating dairy cows. *J. Dairy Sci.* 94:793-799.

426 Beutler, B. 2004. Inferences, questions and possibilities in Toll-like receptor signalling. *Nature*
427 430:257-263.

428 Bicalho, M. L. S., V. S. Machado, G. Oikonomou, R. O. Gilbert, and R. C. Bicalho. 2012.
429 Association between virulence factors of *Escherichia coli*, *Fusobacterium necrophorum*,
430 and *Arcanobacterium pyogenes* and uterine diseases of dairy cows. *Vet. Microbiol.*
431 157:125-131.

432 Bicalho, R. C. et al. 2010. Molecular and epidemiological characterization of bovine intrauterine
433 *Escherichia coli*. *J. Dairy Sci.* 93:5818-5830.

434 Bondurant, R. H. 1999. Inflammation in the bovine female reproductive tract. *J. Anim. Sci.* 77
435 Suppl 2:101-110.

436 Bonnett, B. N., S. W. Martin, V. P. Gannon, R. B. Miller, and W. G. Etherington. 1991.
437 Endometrial biopsy in Holstein-Friesian dairy cows. III. Bacteriological analysis and
438 correlations with histological findings. *Can. J. Vet. Res.* 55:168-173.

439 Borges, A. M., G. D. Healey, and I. M. Sheldon. 2012. Explants of intact endometrium to model
440 bovine innate immunity and inflammation ex vivo. *Am. J. Reprod. Immuno.* 67:526-539.

441 Borsberry, S., and H. Dobson. 1989. Periparturient diseases and their effect on reproductive
442 performance in five dairy herds. *Vet. Rec.* 124:217-219.

443 Bromfield, J. J., and I. M. Sheldon. 2011. Lipopolysaccharide initiates inflammation in bovine
444 granulosa cells via the TLR4 pathway and perturbs oocyte meiotic progression in vitro.
445 *Endocrinology* 152:5029-5040.

446 Bromfield, J. J., and I. M. Sheldon. 2013. Lipopolysaccharide reduces the primordial follicle
447 pool in the bovine ovarian cortex ex vivo and in the murine ovary in vivo. *Biol. Reprod.*
448 88:98.

449 Bruun, J., A. K. Ersboll, and L. Alban. 2002. Risk factors for metritis in Danish dairy cows.
 450 Prev. Vet. Med. 54:179-190.

451 Bychkov, V. 1990. Ovarian pathology in chronic pelvic inflammatory disease. Gynecol. Obstet.
 452 Invest. 30:31-33.

453 Castrillon, D. H., L. Miao, R. Kollipara, J. W. Horner, and R. A. DePinho. 2003. Suppression of
 454 ovarian follicle activation in mice by the transcription factor Foxo3a. Science 301:215-
 455 218.

456 Chalfie, M., Y. Tu, G. Euskirchen, W. W. Ward, and D. C. Prasher. 1994. Green fluorescent
 457 protein as a marker for gene expression. Science 263:802-805.

458 Chegini, N., C. Ma, M. Roberts, R. S. Williams, and B. A. Ripps. 2002. Differential expression
 459 of interleukins (IL) IL-13 and IL-15 throughout the menstrual cycle in endometrium of
 460 normal fertile women and women with recurrent spontaneous abortion. J. Reprod.
 461 Immunol. 56:93-110.

462 Chenault, J. R., J. F. McAllister, S. T. Chester Jr, K. J. Dame, F. M. Kausche, E. J. Robb. 2004.
 463 Efficacy of ceftiofur hydrochloride sterile suspension administered parenterally for the
 464 treatment of acute postpartum metritis in dairy cows. J. Am. Vet. Med. Assoc. 224:1634-
 465 1639.

466 Chien, A., D. B. Edgar, and J. M. Trela. 1976. Deoxyribonucleic acid polymerase from the
 467 extreme thermophile *Thermus aquaticus*. J. Bacteriol. 127:1550-1557.

468 Corbeil, L. B., G. G. Schurig, P. J. Bier, and A. J. Winter. 1975. Bovine venereal vibriosis:
 469 antigenic variation of the bacterium during infection. Infect. Immun. 11:240-244.

470 Cronin, J. G., M. L. Turner, L. Goetze, C. E. Bryant, and I. M. Sheldon. 2012. Toll-like receptor
 471 4 and MYD88-dependent signaling mechanisms of the innate immune system are

472 essential for the response to lipopolysaccharide by epithelial and stromal cells of the
 473 bovine endometrium. *Biol. Reprod.* 86:51.

474 Davies, D., K. G. Meade, S. Herath, P. D. Eckersall, D. Gonzalez, J. O. White, R. S. Conlan, C.
 475 O'Farrelly, I. M. Sheldon. 2008. Toll-like receptor and antimicrobial peptide expression
 476 in the bovine endometrium. *Reprod. Biol. Endocrinol.* 6:53.

477 de Boer, M. W., S. J. LeBlanc, J. Dubuc, S. Meier, W. Heuwieser, S. Arlt, R. O. Gilbert, S.
 478 McDougall. 2014. Invited review: Systematic review of diagnostic tests for reproductive-
 479 tract infection and inflammation in dairy cows. *J. Dairy Sci.* 97:3983-3999.

480 Dohmen, M. J., K. Joop, A. Sturk, P. E. Bols, and J. A. Lohuis. 2000. Relationship between
 481 intra-uterine bacterial contamination, endotoxin levels and the development of
 482 endometritis in postpartum cows with dystocia or retained placenta. *Theriogenology*
 483 54:1019-1032.

484 Dong, J., D. F. Albertini, K. Nishimori, T. R. Kumar, N. Lu, M. M. Matzuk. 1996. Growth
 485 differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 383:531-
 486 535.

487 Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2010. Definitions and
 488 diagnosis of postpartum endometritis in dairy cows. *J. Dairy Sci.* 93:5225-5233.

489 Espey, L. L. 1980. Ovulation as an inflammatory reaction--a hypothesis. *Biol. Reprod.* 22:73-
 490 106.

491 Fischer, C., M. Drillich, S. Odau, W. Heuwieser, R. Einspanier, C. Gabler. 2010. Selected pro-
 492 inflammatory factor transcripts in bovine endometrial epithelial cells are regulated during
 493 the oestrous cycle and elevated in case of subclinical or clinical endometritis. *Reprod.*
 494 *Fertil. Dev.* 22:818-829.

495 Fortune, J. E. 2003. The early stages of follicular development: activation of primordial follicles
 496 and growth of preantral follicles. *Anim. Reprod. Sci.* 78:135-163.

497 Galvao, K. N., L. F. Greco, J. M. Vilela, M. F. Sa Filho, and J. E. Santos. 2009. Effect of
 498 intrauterine infusion of ceftiofur on uterine health and fertility in dairy cows. *J. Dairy Sci.*
 499 92:1532-1542.

500 Giuliadori, M. J., R. P. Magnasco, D. Becu-Villalobos, I. M. Lacau-Mengido, C. A. Risco, R. L.
 501 de la Sota. 2013. Metritis in dairy cows: risk factors and reproductive performance. *J.*
 502 *Dairy Sci.* 96:3621-3631.

503 Goldstone, R. J., M. Amos, R. Talbot, H. J. Schuberth, O. Sandra, I. M. Sheldon, D. G. Smith.
 504 2014a. Draft Genome Sequence of *Trueperella pyogenes*, Isolated from the Infected
 505 Uterus of a Postpartum Cow with Metritis. *Genome Announc.* 2:e00194-14.

506 Goldstone, R. J., R. Talbot, H. J. Schuberth, O. Sandra, I. M. Sheldon, D. G. Smith. 2014b. Draft
 507 Genome Sequence of *Escherichia coli* MS499, Isolated from the Infected Uterus of a
 508 Postpartum Cow with Metritis. *Genome Announc.* 2:e00217-14.

509 Goyette, J., and C. L. Geczy. 2011. Inflammation-associated S100 proteins: new mechanisms
 510 that regulate function. *Amino Acids* 41:821-842.

511 Hansen, P. J., K. B. Dobbs, and A. C. Denicol. 2014. Programming of the preimplantation
 512 embryo by the embryokine colony stimulating factor 2. *Anim. Reprod. Sci.* 149:59-66.

513 Haziak, K., A. P. Herman, and D. Tomaszewska-Zaremba. 2014. Effects of central injection of
 514 anti-LPS antibody and blockade of TLR4 on GnRH/LH secretion during immunological
 515 stress in anestrus ewes. *Mediators Inflamm.* 2014:867170.

516 Herath, S., D. P. Fischer, D. Werling, E. J. Williams, S. T. Lilly, H. Dobson, C. E. Bryant, I. M.
 517 Sheldon. 2006. Expression and function of Toll-like receptor 4 in the endometrial cells of
 518 the uterus. *Endocrinology* 147:562-570.

519 Herath, S., S. T. Lilly, D. P. Fischer, E. J. Williams, H. Dobson, C. E. Bryant, I. M. Sheldon.
 520 2009a. Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin
 521 F2alpha to prostaglandin E2 in bovine endometrium. *Endocrinology* 150:1912-1920.

522 Herath, S., S. T. Lilly, N. R. Santos, R. O. Gilbert, L. Goetze, C. E. Bryant, J. O. White, J.
 523 Cronin, I. M. Sheldon. 2009b. Expression of genes associated with immunity in the
 524 endometrium of cattle with disparate postpartum uterine disease and fertility. *Reprod.*
 525 *Biol. Endocrinol.* 7:55.

526 Herath, S., E. J. Williams, S. T. Lilly, R. O. Gilbert, H. Dobson, C. E. Bryant, I. M. Sheldon.
 527 2007. Ovarian follicular cells have innate immune capabilities that modulate their
 528 endocrine function. *Reproduction* 134:683-693.

529 Hill, J., and R. Gilbert. 2008. Reduced quality of bovine embryos cultured in media conditioned
 530 by exposure to an inflamed endometrium. *Aust. Vet. J.* 86:312-316.

531 Jones, G. R., and C. G. Gemmell. 1982. Impairment by *Bacteroides* species of opsonisation and
 532 phagocytosis of enterobacteria. *J. Med. Microbiol* 15:351-361.

533 Karsch, F. J., D. F. Battaglia, K. M. Breen, N. Debus, and T. G. Harris. 2002. Mechanisms for
 534 ovarian cycle disruption by immune/inflammatory stress. *Stress* 5:101-112.

535 Kasimanickam, R., T. F. Duffield, R. A. Foster, C. J. Gartley, K. E. Leslie, J. S. Walton, W. H.
 536 Johnson. 2004. Endometrial cytology and ultrasonography for the detection of subclinical
 537 endometritis in postpartum dairy cows. *Theriogenology* 62:9-23.

538 Kawai, T., and S. Akira. 2010. The role of pattern-recognition receptors in innate immunity:
539 update on Toll-like receptors. *Nature Immunol.* 11:373-384.

540 Krogh, A. 1929. The progress of physiology. *Science* 70:200-204.

541 Laird, M. H., S. H. Rhee, D. J. Perkins, A. E. Medvedev, W. Piao, M. J. Fenton, S. N. Vogel.
542 2009. TLR4/MyD88/PI3K interactions regulate TLR4 signaling. *J. Leukoc. Biol* 85:966-
543 977.

544 Lavon, Y., G. Leitner, T. Goshen, R. Braw-Tal, S. Jacoby, D. Wolfenson. 2008. Exposure to
545 endotoxin during estrus alters the timing of ovulation and hormonal concentrations in
546 cows. *Theriogenology* 70:956-967.

547 LeBlanc, S. J., T. F. Duffield, K. E. Leslie, K. G. Bateman, G. P. Keefe, J. S. Walton, W. H.
548 Johnson. 2002. Defining and diagnosing postpartum clinical endometritis and its impact
549 on reproductive performance in dairy cows. *J. Dairy Sci.* 85:2223-2236.

550 Lemaitre, B., E. Nicolas, L. Michaut, J. M. Reichhart, and J. A. Hoffmann. 1996. The
551 dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal
552 response in *Drosophila* adults. *Cell* 86:973-983.

553 Li, R., X. Luo, Q. Pan, I. Zineh, D. F. Archer, R. S. Williams, N. Chegini. 2006. Doxycycline
554 alters the expression of inflammatory and immune-related cytokines and chemokines in
555 human endometrial cells: implication in irregular uterine bleeding. *Hum. Reprod.*
556 21:2555-2563.

557 Machado, V. S., M. L. Bicalho, E. B. Meira Junior, R. Rossi, B. L. Ribeiro, S. Lima, T. Santos,
558 A. Kussler, C. Foditsch, E. K. Ganda, G. Oikonomou, S. H Cheong, R. O. Gilbert, R. C.
559 Bicalho. 2014. Subcutaneous immunization with inactivated bacterial components and

560 purified protein of *Escherichia coli*, *Fusobacterium necrophorum* and *Trueperella*
 561 *pyogenes* prevents puerperal metritis in Holstein dairy cows. *PLoS One* 9:e91734.
 562 Machado, V. S., G. Oikonomou, M. L. Bicalho, W. A. Knauer, R. Gilbert, R. C. Bicalho. 2012.
 563 Investigation of postpartum dairy cows uterine microbial diversity using metagenomic
 564 pyrosequencing of the 16S rRNA gene. *Vet. Microbiol.* 159:460-469.
 565 Magata, F., M. Horiuchi, R. Echizenya, R. Miura, S. Chiba, M. Matsui, A. Miyamoto, Y.
 566 Kobayashi, T. Shimizu. 2014. Lipopolysaccharide in ovarian follicular fluid influences
 567 the steroid production in large follicles of dairy cows. *Anim. Reprod. Sci.* 144:6-13.
 568 Margolis, R. C. 1976. Ovarian histology in pelvic inflammatory disease. *J. Am. Osteopath.*
 569 *Assoc.* 75:602-605.
 570 Markusfeld, O. 1987. Periparturient traits in seven high dairy herds. Incidence rates, association
 571 with parity, and interrelationships among traits. *J. Dairy Sci.* 70:158-166.
 572 Mateus, L., L. L. da Costa, F. Bernardo, and J. R. Silva. 2002. Influence of puerperal uterine
 573 infection on uterine involution and postpartum ovarian activity in dairy cows. *Reprod.*
 574 *Domest. Anim.* 37:31-35.
 575 Mathis, D., and S. E. Shoelson. 2011. Immunometabolism: an emerging frontier. *Nat. Rev.*
 576 *Immunol.* 11:81.
 577 McDougall, S. 2001. Effect of intrauterine antibiotic treatment on reproductive performance of
 578 dairy cows following periparturient disease. *N. Z. Vet. J.* 49:150-158.
 579 McGregor, J. A., D. Lawellin, A. Franco-Buff, J. K. Todd, and E. L. Makowski. 1986. Protease
 580 production by microorganisms associated with reproductive tract infection. *Am. J.*
 581 *Obstet. Gynecol.* 154:109-114.

582 Miller, A. N., E. J. Williams, K. Sibley, S. Herath, E. A. Lane, J. Fishwick, D. M. Nash, A. N.
 583 Rycroft, H. Dobson, C. E. Bryant, I. M. Sheldon. 2007. The effects of *Arcanobacterium*
 584 *pyogenes* on endometrial function in vitro, and on uterine and ovarian function in vivo.
 585 *Theriogenology* 68:972-980.

586 Molyneaux, K. A., K. Schaible, and C. Wylie. 2003. GP130, the shared receptor for the LIF/IL6
 587 cytokine family in the mouse, is not required for early germ cell differentiation, but is
 588 required cell-autonomously in oocytes for ovulation. *Development* 130:4287-4294.

589 Narayanan, S., G. C. Stewart, M. M. Chengappa, L. Willard, W. Shuman, M. Wilkerson, T. G.
 590 Nagaraja. 2002. *Fusobacterium necrophorum* leukotoxin induces activation and apoptosis
 591 of bovine leukocytes. *Infect. Immun.* 70:4609-4620.

592 Okumura, E., T. Fukuhara, H. Yoshida, S. Hanada Si, R. Kozutsumi, M. Mori, K. Tachibana, T.
 593 Kishimoto. 2002. Akt inhibits Myt1 in the signalling pathway that leads to meiotic
 594 G2/M-phase transition. *Nat. Cell Biol.* 4:111.

595 Olson, J. D., L. Ball, R. G. Mortimer, P. W. Farin, W. S. Adney, E. M. Huffman. 1984. Aspects
 596 of bacteriology and endocrinology of cows with pyometra and retained fetal membranes.
 597 *Am. J. Vet. Res.* 45:2251-2255.

598 Opsomer, G., Y. T. Gröhn, J. Hertl, M. Coryn, H. Deluyker, A. de Kruif. 2000. Risk factors for
 599 post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study.
 600 *Theriogenology* 53:841-857.

601 Parsonson, I. M., B. L. Clark, and J. H. Dufty. 1976. Early pathogenesis and pathology of
 602 *Tritrichomonas foetus* infection in virgin heifers. *J. Comp. Pathol.* 86:59-66.

603 Peter, A. T., W. T. Bosu, and R. J. DeDecker. 1989. Suppression of preovulatory luteinizing
604 hormone surges in heifers after intrauterine infusions of *Escherichia coli* endotoxin. *Am.*
605 *J. Vet. Res.* 50:368-373.

606 Pinedo, P. J., K. N. Galvao, and C. M. Seabury. 2013. Innate immune gene variation and
607 differential susceptibility to uterine diseases in Holstein cows. *Theriogenology* 80:384-
608 390.

609 Poltorak, A., X. He, I. Smirnova, M. Y. Liu, C. Van Huffel, X. Du, D. Birdwell, E. Alejos, M.
610 Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton, B. Beutler. 1998.
611 Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene.
612 *Science* 282:2085-2088.

613 Potter, T. J., J. Guitian, J. Fishwick, P. J. Gordon, and I. M. Sheldon. 2010. Risk factors for
614 clinical endometritis in postpartum dairy cattle. *Theriogenology* 74:127-134.

615 Price, J. C., J. J. Bromfield, and I. M. Sheldon. 2013. Pathogen-associated molecular patterns
616 initiate inflammation and perturb the endocrine function of bovine granulosa cells from
617 ovarian dominant follicles via TLR2 and TLR4 pathways. *Endocrinology* 154:3377-
618 3386.

619 Price, J. C., J. Cronin, and I. M. Sheldon. 2012. Toll-like receptor expression and function in the
620 COV434 granulosa cell line. *Am. J. Reprod. Immunol.* 68:205-217.

621 Price, J. C., and I. M. Sheldon. 2013. Granulosa cells from emerged antral follicles of the bovine
622 ovary initiate inflammation in response to bacterial pathogen-associated molecular
623 patterns via Toll-like receptor pathways. *Biol. Reprod.* 89:119.

624 Prunner, I. H. Pothmann, K. Wagener, M. Giuliadori, J. Huber, M. Ehling-Schulz, M. Drillich.
 625 2014. Dynamics of bacteriologic and cytologic changes in the uterus of postpartum dairy
 626 cows. *Theriogenology*.
 627 Rasweiler, J. J. 4th., and H. de Bonilla. 1992. Menstruation in short-tailed fruit bats (*Carollia*
 628 spp.). *J. Reprod. Fertil.* 95:231-248.
 629 Reddy, P., L. Liu, D. Adhikari, K. Jagarlamudi, S. Rajareddy, Y. Shen, C. Du, W. Tang, T.
 630 Hämäläinen, S. L. Peng, Z. J. Lan, A. J. Cooney, I. Huhtaniemi, K. Liu. 2008. Oocyte-
 631 specific deletion of *Pten* causes premature activation of the primordial follicle pool.
 632 *Science* 319:611-613.
 633 Ribeiro, E. S., F. S. Lima, L. F. Greco, R. S. Bisinotto, A. P. Monteiro, M. Favoreto, H. Ayres,
 634 R. S. Marsola, N. Martinez, W. W. Thatcher, J. E. Santos. 2013. Prevalence of
 635 periparturient diseases and effects on fertility of seasonally calving grazing dairy cows
 636 supplemented with concentrates. *J. Dairy Sci.* 96:5682-5697.
 637 Richards, J. S., Z. Liu, and M. Shimada. 2008. Immune-like mechanisms in ovulation. *Trends*
 638 *Endocrinol. Metab.* 19:191-196.
 639 Ross, J. D. 2002. An update on pelvic inflammatory disease. *Sex Transm. Infect.* 78:18-19.
 640 Rowson, L. E., G. E. Lamming, and R. M. Fry. 1953. Influence of ovarian hormones on uterine
 641 infection. *Nature* 171:749-750.
 642 Sancho-Tello M., T. Y. Chen, T. K. Clinton, R. Lyles, R. F. Moreno, L. Tilzer, K. Imakawa, and
 643 P. F. Terranova. 1992. Evidence for lipopolysaccharide binding in human granulosa-
 644 luteal cells. *J. Endocrinol.* 135:571-578.

645 Sannmann, I., S. Arlt, and W. Heuwieser. 2012. A critical evaluation of diagnostic methods used
646 to identify dairy cows with acute post-partum metritis in the current literature. *J. Dairy*
647 *Res.* 79:436-444.

648 Schurig, G. G., C. E. Hall, L. B. Corbell, J. R. Duncan, and A. J. Winter. 1975. Bovine venereal
649 vibriosis: cure of genital infection in females by systemic immunization. *Infect. Immun.*
650 11:245-251.

651 Sheldon, I. M., J. Cronin, L. Goetze, G. Donofrio, and H. J. Schuberth. 2009. Defining
652 postpartum uterine disease and the mechanisms of infection and immunity in the female
653 reproductive tract in cattle. *Biol. Reprod.* 81:1025-1032.

654 Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine
655 disease in cattle. *Theriogenology* 65:1516-1530.

656 Sheldon, I. M., D. E. Noakes, A. Rycroft, and H. Dobson. 2001. Acute phase protein responses
657 to uterine bacterial contamination in cattle after calving. *Vet. Rec.* 148:172-175.

658 Sheldon, I. M., D. E. Noakes, A. N. Rycroft, D. U. Pfeiffer, and H. Dobson. 2002. Influence of
659 uterine bacterial contamination after parturition on ovarian dominant follicle selection
660 and follicle growth and function in cattle. *Reproduction* 123:837-845.

661 Sheldon, I. M., and M. H. Roberts. 2010. Toll-like receptor 4 mediates the response of epithelial
662 and stromal cells to lipopolysaccharide in the endometrium. *PLoS One* 5:e12906.

663 Sheldon, I. M., A. N. Rycroft, B. Dogan, M. Craven, J. J. Bromfield, A. Chandler, M. H.
664 Roberts, S. B. Price, R. O. Gilbert, K. W. Simpson. 2010. Specific strains of *Escherichia*
665 *coli* are pathogenic for the endometrium of cattle and cause pelvic inflammatory disease
666 in cattle and mice. *PLoS One* 5:e9192.

667 Shimada, M., I. Hernandez-Gonzalez, I. Gonzalez-Robanya, and J. S. Richards. 2006. Induced
 668 expression of pattern recognition receptors in cumulus oocyte complexes: novel evidence
 669 for innate immune-like functions during ovulation. *Mol. Endocrinol.* 20:3228-3239.

670 Shimada, M., Y. Yanai, T. Okazaki, N. Noma, I. Kawashima, T. Mori, J. S. Richards. 2008.
 671 Hyaluronan fragments generated by sperm-secreted hyaluronidase stimulate
 672 cytokine/chemokine production via the TLR2 and TLR4 pathway in cumulus cells of
 673 ovulated COCs, which may enhance fertilization. *Development* 135:2001-2011.

674 Silvestre, F. T., T. S. Carvalho, P. C. Crawford, J. E. Santos, C. R. Staples, T. Jenkins, W. W.
 675 Thatcher. 2011. Effects of differential supplementation of fatty acids during the
 676 peripartum and breeding periods of Holstein cows: II. Neutrophil fatty acids and function,
 677 and acute phase proteins. *J. Dairy Sci.* 94:2285-2301.

678 Skirrow, S. Z., and R. H. BonDurant. 1990. Induced *Tritrichomonas foetus* infection in beef
 679 heifers. *J. Am. Vet. Med. Assoc.* 196:885-889.

680 Soto, P., R. P. Natzke, and P. J. Hansen. 2003. Identification of possible mediators of embryonic
 681 mortality caused by mastitis: actions of lipopolysaccharide, prostaglandin F2alpha, and
 682 the nitric oxide generator, sodium nitroprusside dihydrate, on oocyte maturation and
 683 embryonic development in cattle. *Am. J. Reprod. Immunol.* 50:263-272.

684 Spaniel-Borowski, K. 2011. Ovulation as danger signaling event of innate immunity. *Mol. Cell.*
 685 *Endocrinol.* 333:1-7.

686 Spicer, L. J. 1998. Tumor necrosis factor-alpha (TNF-alpha) inhibits steroidogenesis of bovine
 687 ovarian granulosa and thecal cells in vitro. Involvement of TNF-alpha receptors.
 688 *Endocrine* 8:109-115.

689 Spicer, L. J., P. Y. Aad, D. Allen, S. Mazerbourg, and A. J. Hsueh. 2006. Growth differentiation
690 factor-9 has divergent effects on proliferation and steroidogenesis of bovine granulosa
691 cells. *J. Endocrinol.* 189:329-339.

692 Spicer, L. J., S. E. Echternkamp, S. F. Canning, and J. M. Hammond. 1988. Relationship
693 between concentrations of immunoreactive insulin-like growth factor-I in follicular fluid
694 and various biochemical markers of differentiation in bovine antral follicles. *Biol.*
695 *Reprod.* 39:573-580.

696 Suzuki, C., K. Yoshioka, S. Iwamura, and H. Hirose. 2001. Endotoxin induces delayed ovulation
697 following endocrine aberration during the proestrous phase in Holstein heifers. *Domest.*
698 *Anim. Endocrinol.* 20:267-278.

699 Swangchan-Uthai, T., C. R. Lavender, Z. Cheng, A. A. Fouladi-Nashta, and D. C. Wathes. 2012.
700 Time course of defense mechanisms in bovine endometrium in response to
701 lipopolysaccharide. *Biol. Reprod.* 87:135.

702 Turner, M. L., J. G. Cronin, G. D. Healey, and I. M. Sheldon. 2014. Epithelial and stromal cells
703 of bovine endometrium have roles in innate immunity and initiate inflammatory
704 responses to bacterial lipopeptides in vitro via Toll-like receptors TLR2, TLR1, and
705 TLR6. *Endocrinology* 155:1453-1465.

706 Van Slyke, P., M. L. Coll, Z. Master, H. Kim, J. Filmus, D. J. Dumont. 2005. Dok-R mediates
707 attenuation of epidermal growth factor-dependent mitogen-activated protein kinase and
708 Akt activation through processive recruitment of c-Src and Csk. *Mol. Cell. Biol.* 25:3831-
709 3841.

710 Vieira-Neto, A., R. O. Gilbert, W. R. Butler, J. E. Santos, E. S. Ribeiro, M. M. Vercouteren, R.
711 G. Bruno, J. H. Bittar, K. N. Galvão. 2014. Individual and combined effects of

712 anovulation and cytological endometritis on the reproductive performance of dairy cows.
 713 J. Dairy Sci. 97:5415-5425.

714 Wagener, K., T. Grunert, I. Prunner, M. Ehling-Schulz, and M. Drillich. 2014. Dynamics of
 715 uterine infections with *Escherichia coli*, *Streptococcus uberis* and *Trueperella pyogenes*
 716 in post-partum dairy cows and their association with clinical endometritis. Vet. J. (in
 717 press).

718 Wandji, S. A., V. Srsen, A. K. Voss, J. J. Eppig, and J. E. Fortune. 1996. Initiation in vitro of
 719 growth of bovine primordial follicles. Biol. Reprod. 55:942-948.

720 Wathes, D. C., Z. Cheng, W. Chowdhury, M. A. Fenwick, R. Fitzpatrick, D. G. Morris, J. Patton,
 721 J. J. Murphy 2009. Negative energy balance alters global gene expression and immune
 722 responses in the uterus of postpartum dairy cows. Physiol. Genomics 39:1-13.

723 Weiner, S., and E. E. Wallach. 1974. Ovarian histology in pelvic inflammatory disease. Obstet.
 724 Gynecol. 43:431-437.

725 Williams, E. J. et al. 2007. The relationship between uterine pathogen growth density and
 726 ovarian function in the postpartum dairy cow. Theriogenology 68:549-559.

727 Williams, E. J., D. P. Fischer, D. E. Noakes, G. C. England, A. Rycroft, H. Dobson, I. M.
 728 Sheldon. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial
 729 infection and the immune response in cattle. Theriogenology 63:102-117.

730 Yan, C., P. Wang, J. DeMayo, F. J. DeMayo, J. A. Elvin, C. Carino, S. V. Prasad, S. S. Skinner,
 731 B. S. Dunbar, J. L. Dube, A. J. Celeste, M. M. Matzuk. 2001. Synergistic roles of bone
 732 morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. Mol.
 733 Endocrinol. 15:854-866.

734 Zwald, N. R., K. A. Weigel, Y. M. Chang, R. D. Welper, and J. S. Clay. 2004. Genetic selection
735 for health traits using producer-recorded data. I. Incidence rates, heritability estimates,
736 and sire breeding values. J. Dairy Sci. 87:4287-4294.

737

739 **Table 1.** Toll-like receptor ligands and endometrial expression.

Toll-like receptor	Ligand	Uterine expression	Endometrial cell type
<i>TLR1</i>	Bacterial lipoproteins	NP, postpartum	Epi, stroma
<i>TLR2</i>	Peptidoglycan, lipoteichoic acid and lipoprotein (most diverse)	NP, postpartum (↑ in infertile animals*)	Epi, stroma
<i>TLR3</i>	Double stranded RNA	NP, postpartum	Epi, stroma
<i>TLR4</i>	Lipopolysaccharide	NP, postpartum (↑ in infertile animals*)	Epi, stroma
<i>TLR5</i>	Flagellin	NP, postpartum	Epi
<i>TLR6</i>	Lipoteichoic acid, lipoproteins	NP, postpartum	Epi, stroma
<i>TLR7</i>	Single stranded RNA	NP, postpartum	Epi, stroma
<i>TLR8</i>	Single stranded RNA	NP, postpartum	ND
<i>TLR9</i>	Unmethylated CpG DNA	NP, postpartum	Epi, stroma
<i>TLR10</i>	Unknown	NP, postpartum	Stroma

740 Exogenous ligands for the various TLR, their described expression in either non-pregnant or
741 postpartum uterus biopsy, and cellular expression in purified endometrial epithelium or stroma.
742 Abbreviations: NP = non-pregnant; Epi = epithelium; ND = not detected; * denotes an increased
743 expression in infertile compared to fertile animals. Information is derived from (Davies et al.,
744 2008; Herath et al., 2009b; Kawai and Akira, 2010).

745

746

FIGURE CAPTIONS

747 **Figure 1.** Schematic representation of uterine infection and impacts on the reproductive tract.
748 This figure represents the all-encompassing effects of uterine bacterial infection on
749 neuroendocrine signaling, uterine health and ovarian function. Brain; GnRH and LH production
750 are reduced. Endometrium; bacterial pyolysin (**PLO**) disrupts endometrial cells by osmotic lysis,
751 while lipopolysaccharides (**LPS**) initiates an inflammatory response via Toll-like receptor (**TLR**)
752 4 activation increasing cytokine, chemokine and PGE₂ production. Ovary; the primordial follicle
753 reserve is depleted, follicle growth is retarded and luteal phase prolonged. Ovarian granulosa
754 cells respond to bacterial LPS in a TLR4 dependent manner increasing inflammatory mediators,
755 reducing aromatase and estradiol, and reducing oocyte competence. Illustration by Stacey Jones,
756 UF/IFAS.

757 **Figure 2.** Schematic representation of the redundancies between Toll-like receptor (**TLR**) 4/IL-
758 6 signaling and the cellular pathways in granulosa cells and oocytes involved in primordial
759 follicle activation. The left panel shows the intracellular pathways of TLR4 or IL-6 activation
760 through phosphatidylinositol-4,5-bisphosphate 3-kinase (**PI3K**) and Protein kinase B (**AKT**).
761 The right panel shows the process of primordial follicle activation utilizing the same PI3K and
762 AKT pathway which is regulated by the balance of phosphatase and tensin homolog (**PTEN**)
763 activation by tyrosine kinase receptors. We propose that bacterial activation of the TLR/IL-6
764 pathway (left) contributes to inappropriate activation of the follicle activation pathway (right).

Figure 1

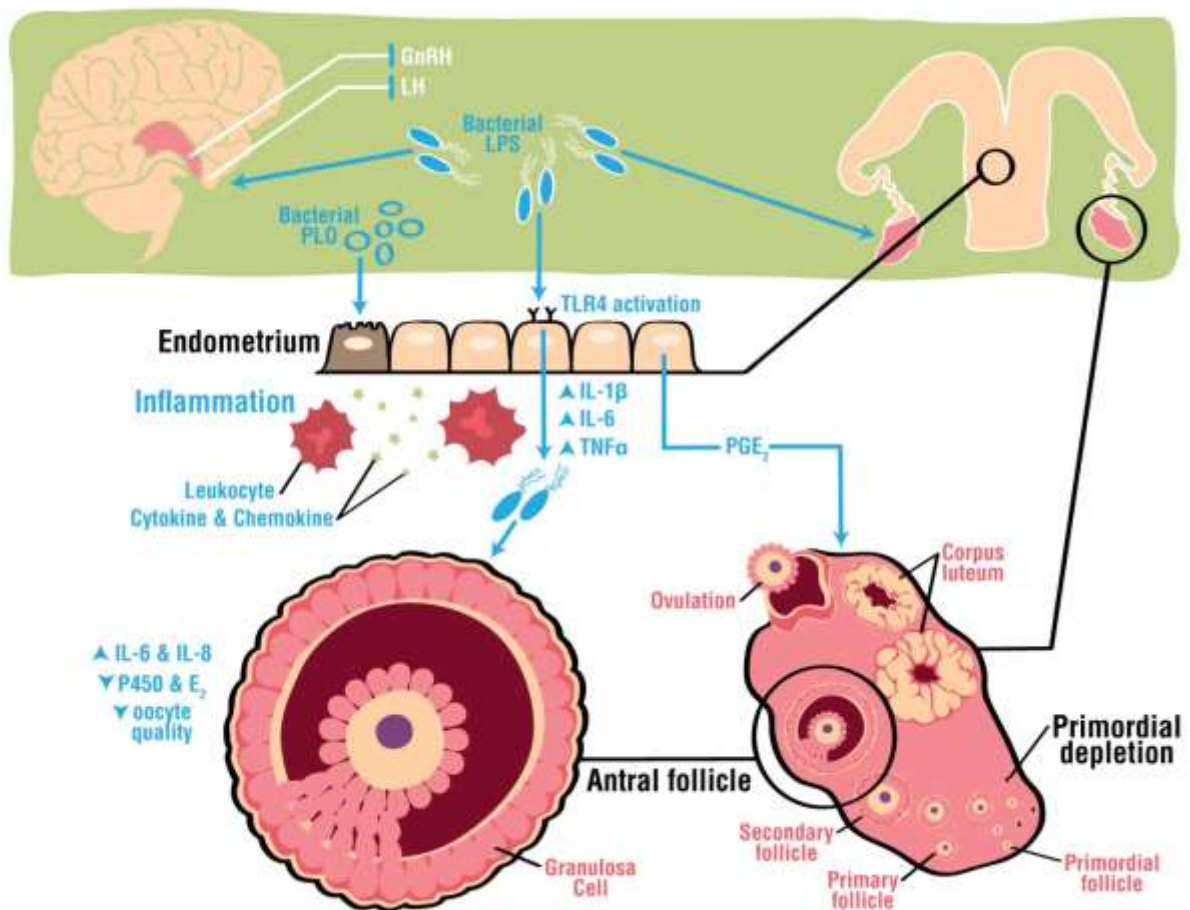


Figure 2

